Leishmania and DC2.4 cells culture

In our project, we used wild-type clone 12-1 of leishmania amazonensis (RAT/BA/74/LV78) which doubly transfected with pX-alad and p6.5-pbgd (DT; 8–10) as model. Leishmania were routinely grown as promastigotes at 25 ^{o}C in medium 199 (Sigma) supplemented with 10% heat-inactivated fetal bovine serum (HIFBS) and 25mM HEPES to pH 7.4. Through two different antibiotics, G418 (100 μg/ml) and tunicamycin (20 μg/ml) to select the correct content of PBGD and ALAD, which are third and second enzymes in the heme biosynthetic pathway. The cells need to be passaged in a week or when reached the peak cell density of ~5x10^{7} cells/ml at the stationary phase. Use T25 flask at 4ml/flask with solid screw-cap tightened to expand the cells. Stock cultures were set up by inoculation of a turbid culture at one drop (~50 μl) per ml into the 15 ml culture tubes. Before exposure of these DT cultures to ALA for inducing cytoplasmic accumulation of uroporphyrin 1 (URO), they were grown in drug-free medium to stationary phase to avoid the potential cytotoxicity of the drugs effect carried over to the host cells.

DC2.4 line, mouse dendritic cells were grown in RPMI-1640 supplemented with 10% FBS at 37 ^{o}C, 5% CO_2 incubator.